Soybean oligosaccharides alter colon short-chain fatty acid production and microbial population in vitro

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ABSTRACT: This study was conducted to determine fermentation characteristics of soybean oligosaccharides (SBOS) in an in vitro system. Digesta collected from the colon of Huanjiang mini-pigs was used as inoculums, and SBOS (0.2 g per 10 mL fermentation broth) was used as substrate during the in vitro fermentation. The inoculum or inoculum + glucose (0.2 g) was used as negative or positive control, respectively. The slurry was fermented in an anaerobic chamber and gas production (GP) recording was taken after 48 h of incubation by referring to the moving scale on the plunger of the glass syringes, and then GP parameters, pH value, NH₃-N content, short chain fatty acid (SCFA) levels, and microbial community in the fermentation broth were determined. For gas production parameters, pH, and fermentation product determination after 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36, 42, and 48 h of incubation, SBOS and glucose demonstrated similar responses compared to control including increase maximal gas production, decreased lag time, decreased pH, and accumulation of NH₃ and increased SCFA. The exception was rate of GP, which was higher \((P < 0.05)\) for SBOS compared with glucose. Incubation with SBOS increased \((P < 0.05)\) the microbial diversity and population of \(Bifidobacterium\) and \(Lactobacillus\) but decreased \((P < 0.05)\) \(Escherichia\) and \(Streptococcus\) when compared with incubation with glucose. These findings suggested that the SBOS can improve the gut microbiota balance in colon and modulate its metabolism.

Key words: pigs, short chain fatty acids, soybean oligosaccharides

INTRODUCTION

The lumen of the gut, especially large intestine, contains billions of microbes. These microbes use the food residues and endogenous secretions entering from the small intestine and convert them into useful nutrients, such as short-chain fatty acids (SCFA), which can then be absorbed in the large intestine and used by the animal (Hooper et al., 2002). Soybean oligosaccharides (SBOS), as important nutritional components of soybean (\(Glycine max\)), have been reported to have prebiotic properties and approved as generally recognized safe material in the United States (Gatesoupe, 1999). Our previous study indicated that dietary supplementation with SBOS decreased the plasma contents of urea N and NH₃ but increased total protein and some amino acids in Huanjiang mini-pigs (Zhou et al., 2012). In order to explore their regulating roles in gut metabolism, this study was designed to investigate the effects of SBOS on the SCFA production and microbial population in colon using an in vitro gas production (GP) technique.

MATERIALS AND METHODS

Experimental Design

Colon contents were collected from 3 Huanjiang mini-pigs with BW of ~25 kg (fed a normal diet without antibiotic) and used separately as inoculum. The SBOS (stachyose + rafnose \(\geq 85\%\)) was used as substrate. The oven-dried SBOS sample (200 mg) was weighed in 3 replicates into 100 mL glass syringes containing 10 mL carbonate–phosphate buffer solution and incubated...
in a shaking water bath at 37°C for 48 h. These syringes containing only 10 mL of the inoculum or inoculum + 200 mg glucose were used as negative or positive control, respectively. Blanks containing substrates but no inoculum were also prepared to ensure that selected bacterial species had originated from the intestinal contents and not from the substrate (Kong et al., 2009).

Measurement of Gas Production and Analyses

The slurry was fermented in an anaerobic chamber and GP reading was taken after 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36, 42, and 48 h of incubation using moving scale on the plunger of the syringes. Fermentation was terminated by instantaneous freezing in dry ice. To describe the dynamics of in vitro GP over time, the Gompertz function was used (Schofield et al., 1994). The theoretical maximum GP (mL), maximum rate (mL/h), and lag time of GP were calculated using NLREG (version 5.0) software (Sherrod et al., 2000).

The syringe contents were homogenized by vortex and centrifuged at 1000 × g for 10 min. The pH of the resulting supernatant fluid was measured by a pH meter and NH3–N content was determined by a UV spectrophotometer at 550 nm. A mixture of supernatant fluid and 25% metaphosphoric acid solution (4:1 mL) was prepared for the determination of SCFA by gas chromatography (Deschner et al., 1990).

The bacterial community diversity in the fermentation broth was determined by terminal restriction fragment length polymorphism (T-RFLP) and real-time fluorescent quantitative PCR analysis as described by Castillo et al. (2007).

Data are expressed as the mean ± SE. Results were statistically analyzed using one-way ANOVA (SAS Institute, Cary, NC). Duncan’s multiple range test was used to compare differences among the treatment groups with P < 0.05 considered statistically significant.

RESULTS AND DISCUSSION

In vitro Colon Gas and Short Chain Fatty Acids Production

In vitro GP parameters usually reflect the characteristics of fermentation process. The maximum volume and rate of GP positively correlation with the substrate digestion, but the lag time of GP has a negative correlation. The maximum volume and rate of GP in SBOS- and glucose-supplemented groups were higher (P < 0.05), but the lag time of GP was lower (P < 0.05) than the control group (Table 1). The rate of GP in SBOS-supplemented group was 1.6-fold that of glucose group (P < 0.05). The GP, proportion of various gases, and rate of GP have a close relationship with the chemical structure of the substrate and microbes (Jensen and Jorgensen, 1994). These findings suggest that SBOS, mainly composed of stachyose and raffinose, might be associated with the higher rate of digestion through an enhancement of microbial degradation.

The fermentation process of carbohydrates provides metabolic end products for the colon and supplies energy for the growth or maintenance of intestinal microflora. Table 2 shows that the SBOS- or glucose-supplemented group increased (P < 0.05) the production of SCFA compared with the control group. The increased SCFA production created a more acidic environment because data in Table 1 showed that both SBOS and glucose decreased (P < 0.05) the pH values of the fermentation broth.

Soybean Oligosaccharides Altered Colon Microbial Flora In vitro

The SBOS group contained a higher number of T-RFs (26) than the glucose group (20), which suggested that the SBOS could increase the complex and diversity of the bacterial populations (data not shown).
of *Bifidobacterium* to total bacteria in the fermentation broth from the SBOS group was higher (*P* < 0.05) but the ratio of *Escherichia coli* and *Streptococcus* to total bacteria were lower (*P* < 0.05) compared with the glucose group (Figure 1). These findings suggested that SBOS can selectively stimulate the growth of beneficial bacteria in intestines. *Bifidobacterium* does not degrade N-containing compounds directly, but the SCFA produced by bacterial fermentation reduces the intestinal pH and then results in a reduced *NH₃*-N level in fermentation broth (Table 1).

In summary, this study suggested that the SBOS can be selectively fermented by colon bacteria and improve the gut microbiota balance.

**Figure 1.** Effects of incubation in soybean oligosaccharides (SBOS group) or in glucose (glucose group) on 16S rRNA levels of microbiota from colon fermentation broth in vitro (n = 3).

**LITERATURE CITED**


